

TeSeE™ SAP Combi Kit

Short Assay Protocol

192 Tests

Ref.: 355-1186

384 Tests

Ref.: 355-1192

768 Tests

Ref.: 355-1191

REAGENT KITS FOR *IN VITRO* PURIFICATION AND DETECTION OF PrP^{Sc}

Within the European Union, this test is approved as rapid test for the BSE and scrapie testing programmes on cattle, sheep and goats which are set up in accordance with Annex III, chapter A to Regulation (EC) No 999/2001.

User's manual

BIO-RAD

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1 - GENERAL INFORMATION

Transmissible Spongiform Encephalopathies (TSE's) are slow degenerative diseases of the central nervous system induced by unconventional transmissible agents (UTAs) routinely called prions.

TSEs are generally classified according to their etiology, as iatrogenic, familial and/or sporadic. Sheep scrapie has been reported in the 18th century and transmission demonstrated (including to goats). However, the modes of contamination within flocks remain obscure. TSEs were also described in deer and elk (chronic wasting disease, CWD) and in cow (Bovine Spongiform Encephalopathy, BSE).

Humans are also susceptible to certain forms of TSE. There is compelling evidence supporting that Bovine Spongiform Encephalopathy (BSE) has passed from cattle to human, probably through consumption of contaminated meat.

In addition to this variant form of Creutzfeldt-Jakob disease (vCJD), other forms in humans include the Kuru and the iatrogenic Creutzfeldt-Jakob disease.

Pure hereditary forms (such as the Gerstmann-Sträussler-Scheinker syndrome [GSS]) and/or sporadic CJD have been demonstrated in humans, but their incidences are low. We do not know if similar sporadic TSE cases exist in animals.

The main characteristics of these diseases are:

- a slowly progressive but always fatal course,
- absence of conventional infectious agents,
- progressive accumulation in the central nervous system of an abnormal isoform of the natural prion protein (PrP) called PrP^{Sc}. This isoform is characterized by particular biochemical properties and especially by an increased resistance to proteases.

The strikingly long incubation period that precedes the neurological symptoms suggests that important events of TSE pathogenesis might take place in extra nervous sites and especially in peripheral lymphoid tissues.

In spite of many unknown and/or uncertainties, the detection of abnormal PrP^{Sc} is now established as the method, to confirm TSE diagnosis. This detection is mainly achieved from post mortal collected nervous tissues.

Abnormal PrP^{Sc} has also been detected in a number of lymphoid tissue and organs: in the germinal centres of spleen, lymph nodes, tonsils, and/or mucosa-associated lymphoid tissue (but at the research area), in animal models or in scrapie sheep, CWD deers and elks and vCJD patients.

Reagent designed by the "Commissariat à l'Énergie Atomique - CEA" (French Atomic Energy Commission), developed, produced and marketed by Bio-Rad, allow PrP^{Sc} detection on samples of nervous tissues taken from animals.

Test performed with the following reagents and accessories:

- TeSeE™ SAP Combi Kit (192 tests)	Ref.: 355-1186
- TeSeE™ SAP Combi Kit (384 tests)	Ref.: 355-1192
- TeSeE™ SAP Combi Kit (768 tests)	Ref.: 355-1191
- Grinding Tubes (384 tubes)	Ref.: 355-1139
- Grinding Tubes (768 tubes)	Ref.: 355-1137
- Calibration syringe and needle (x 200)	Ref.: 355-1174
or Filter plates (x 50)	Ref.: 355-1179
- Deepwell microplates (x 50)	Ref.: 359-0132
- Medium beads (x 2000)	Ref.: 355-1171*

* Only for peripheral tissues.

2 -TeSeE™ SAP Combi Kit

2-1 Principle

The reagents of the TeSeE™ SAP Combi Kit allow purification, concentration, solubilisation and detection of PrP^{Sc} from samples of tissues obtained from infected animals.

The TeSeE™ SAP Assay is an immuno-enzymatic technique (sandwich format) using 2 monoclonal antibodies for the detection of the abnormal prion protein, resistant to proteinase K, in tissues collected from infected animals.

The solid phase is composed of 12 strips of 8 polystyrene wells coated with the first monoclonal antibody. The second monoclonal antibody is bound to peroxidase.

2-2 Samples

- **Bovine:** purification of PrP^{Sc} is performed on samples from Central Nervous System (CNS). The BSE extraction tool (Ref.: 355-1130) can be used to collect brainstem.

Since distribution of PrP^{Sc} is heterogeneous in central nervous system, obex area from brainstem must be preferably sampled for optimal detection.

Sampling syringe (Ref.: 355-1175) allows easy and rapid sampling of obex area in a secure way.

Please refer to sampling protocol for detailed instructions on good sampling procedure.

- **Small ruminants and cervids:** purification of PrP^{Sc} is performed on samples from Central Nervous System (CNS) or peripheral tissues (lymphoid nodes, spleen,...). The small ruminant extraction tool (Ref.: 355-1184) can be used to collect both brainstem and cerebellum.

Since distribution of PrP^{Sc} is heterogeneous in central nervous system, obex area from brainstem must be preferably sampled for optimal detection.

Samples are cut and weighed individually.

Note: other tissues (tonsils, ileum, eyelid...) can be used for research purposes only.

Samples are stored at +2°C to +8°C when purification is performed within 24 hours or can be stored frozen for several months. They can only be submitted to 3 freezing/thawing cycles. If these samples must be transported, they should be packaged in accordance with current local regulations.

2-3 Composition of the TeSeE™ SAP Combi Kits

LABELLING	TYPE OF REAGENTS	PRESENTATION			STORAGE
		355-1186 (192 tests)	355-1192 (384 tests)	355-1191 (768 tests)	
Reagent A	Denaturing solution	1 vial (55 ml)	1 vial (120 ml)	1 vial (240 ml)	+2°C to +8°C
Reagent B	Clarifying solution Colouring: bromophenol blue	1 vial (55 ml)	1 vial (120 ml)	2 vials (120 ml)	+2°C to +8°C
Reagent C	Resolving buffer Colouring: malachite green	1 vial (7 ml)	1 vial (14 ml)	1 vial (28 ml)	+2°C to +8°C
PK	Proteinase K Colouring: phenol red	1 vial (0.5 ml)	2 vials (0.5 ml)	4 vials (0.5 ml)	+2°C to +8°C
R1	Microplate: 12 strips of 8 wells coated with an anti-PrP monoclonal antibody	2 plates	4 plates	8 plates	+2°C to +8°C
R2	Wash solution: 10-fold concentrated Tris-NaCl buffer pH 7.4 Preservative: ProClin™ 300 (0.01%)	1 vial (250 ml)	2 vials (250 ml)	4 vials (250 ml)	+2°C to +25°C
R3	Negative Control: PBS buffer pH 7.2 supplemented with BSA Preservative: ProClin™ 300 (0.1%)	1 vial (4 ml)	2 vials (4 ml)	4 vials (4 ml)	+2°C to +8°C
R4	Positive Control: PBS buffer pH 7.4 supplemented with non infectious synthetic peptide. Lyophilized. Preservative: ProClin™ 300 (0.1%)	1 vial (q.s. 4 ml)	2 vials (q.s. 4 ml)	4 vials (q.s. 4 ml)	+2°C to +8°C
R6	Sample diluent: PBS buffer pH 7.2 supplemented with BSA and phenol red Preservative: ProClin™ 300 (0.1%)	1 vial (35 ml)	1 vial (70 ml)	1 vial (140 ml)	+2°C to +8°C
R7	Conjugate: 10-fold concentrated peroxidase-labelled anti-PrP monoclonal antibody in PBS buffer pH 7.1 solution supplemented with bovine proteins and coloured with phenol red Preservative: ProClin™ 300 (0.1%)	1 vial (2.8 ml)	2 vials (2.8 ml)	4 vials (2.8 ml)	+2°C to +8°C
R8	Peroxidase Substrate Buffer: Solution of citric acid and sodium acetate pH 4.0 containing 0.015% H ₂ O ₂ and 4% dimethylsulfoxide (DMSO)	1 vial (60 ml)	1 vial (120 ml)	2 vials (120 ml)	+2°C to +8°C
R9	Chromogen: Tetramethylbenzidine (TMB) solution	1 vial (5 ml)	1 vial (10 ml)	1 vial (20 ml)	+2°C to +8°C
R10	Stop solution: 1 N sulphuric acid	1 vial (28 ml)	1 vial (56 ml)	1 vial (112 ml)	+2°C to +8°C
	Adhesive films	8	12	16	

The following reagents are generic components: Reagent A, reagent B, sample diluent (R6), wash solution (R2), peroxidase substrate buffer (R8), chromogen (R9) and stop solution (R10). They can be used with all batches of the TeSeE™ SAP Kits.

2-4 Preparation of Reagents

Before use, let the reagents of the TeSeE™ SAP Combi Kits adjust to room temperature (+18°C to +30°C) for 30 minutes.

1 - Ready to use reagents

Reagent A, B, C, the negative control (R3), sample dilution solution (R6) and stop solution (R10) are ready to use.

Microplates (R1):

Before opening the sealed bag with a desiccant, let the microplate adjust to room temperature (+18°C to +30°C) in its protective packaging to avoid any water condensation in the wells. Open at the solder point and immediately return the unused rows to the sachet.

Tightly close the bag after expelling any air, then store at +2°C to +8°C.

2 - Reagents to reconstitute

Proteinase K:

Reagent A is the dilution buffer for proteinase K.

The solution must be prepared in the following way (4 µl of proteinase K in 1 ml of reagent A):

NUMBER OF SAMPLES	REAGENT A	PROTEINASE K
2	1 ml	4 µl
10	3 ml	12 µl
18	5 ml	20 µl
26	7 ml	28 µl
34	9 ml	36 µl
42	11 ml	44 µl
50	13 ml	52 µl
58	15 ml	60 µl
66	17 ml	68 µl
74	19 ml	76 µl
82	21 ml	84 µl
90	23 ml	92 µl

The volumes must be pipetted exactly. The tip containing the PK has to be correctly rinsed by successive aspiration/distribution cycles in reagent A.

After reconstitution, homogenize the solution by successive inversions until you obtain a red homogeneous solution.

Wash solution (R2):

Dilute wash solution R2 to 1/10 in distilled or ultrapure water (example 100 ml of reagent R2 in 900 ml of distilled water).

Positive control (R4):

Gently tap the vial of positive control (R4) on the laboratory bench to detach any substance adherent to the rubber stopper. Open the vial and dissolve the content in 4 ml of diluent R6. Reseal the vial and let stand for approximately 1 minute, homogenizing gently and occasionally to facilitate dissolution.

Conjugate (R7):

Dilute reagent R7 to 1/10 in the freshly reconstituted wash solution (example: 0.1 ml of reagent R7 in 0.9 ml of reconstituted wash solution) bearing in mind that 1 ml of ready-for-use conjugate is sufficient for 1 row. Homogenize gently. Avoid using a vortex agitator.

Enzymatic development solution (R8 + R9):

Dilute reagent R9 to 1/11 in reagent R8 (example: 0.1 ml of reagent R9 in 1 ml of reagent R8) bearing in mind that 1.1 ml of enzymatic revelation solution is sufficient for 1 row.

Homogenize gently. Avoid using a vortex agitator.

2-5 Storage, Shelf-life

Store the TeSeE™ SAP Combi Kits at +2°C to +8°C. All reagents are stable at this temperature until the expiry date indicated on the kit (before and after opening of the vials).

After dilution, the reconstituted proteinase K solution when stored at room temperature (+18°C to +30°C) must be used within 6 hours.

The shelf-lives of the reagents after preparation are as follows:

LABELLING	REAGENT	SHELF-LIFE
R1	Microplate in tightly closed sachet	1 month at +2°C to +8°C
R2	Diluted wash solution	24 hours at room temperature (+18°C to + 30°C) 2 weeks at +2°C to +8°C
R4	Reconstituted positive control	2 hours at room temperature (+18°C to + 30°C) 4 hours at +2°C to +8°C 6 months at -20°C It is recommended to divide the reconstituted solution into 0.5 ml aliquots and to store them immediately at -20°C. Can be submitted to 3 successive freezing/thawing cycles.
R7	Reconstituted conjugate solution (with diluted wash solution)	8 hours at room temperature (+18°C to + 30°C)
R8 + R9	Development solution	6 hours at room temperature (+18°C to + 30°C) always protected from light

2-6 Assay Procedure

For the semi-automatic processing of the purification protocol, please refer to the TeSeE™ NSP operator's manual.

Procedure for manual processing:

1. Sampling:

For peripheral tissues (lymph nodes, spleen,...) insert one medium bead (Ref.: 355-1171) in the grinding tube, before to add the sample.

Take a mass of 350 mg ± 40 mg of nervous tissue (preferably in the obex area) or 200 mg ± 20 mg of peripheral tissue.

Deposit the samples in grinding tubes, close firmly and proceed to the grinding step in the homogenizer (Ribolyser®, TeSeE™ PRECESS 24™ or TeSeE™ PRECESS 48™ systems).

2. Sample grinding:

Place the tubes in the crown of the homogenizer (Ribolyser®, TeSeE™ PRECESS 24™ or TeSeE™ PRECESS 48™ systems). Perform one agitation cycle with the following instrument parameters:

	Ribolyser®		TeSeE™ PRECESS 24™ or 48™	
	Nervous tissues	Peripheral tissues	Nervous tissues	Peripheral tissues
Time (sec.)	45	2 x 45*	-	-
Speed	6.5	6.5	-	-
Program	-	-	Program 1	Program 2

* Wait a 5 minutes pause between the 2 agitation cycles.

When grinding is insufficient, another 1 or 2 agitation cycles can be performed, by ensuring that the temperature of the tube returns to room temperature (+18°C to +30°C) between each cycle (using crushed ice for example).

3. Sample transfer:

Remove the grinding tubes from the homogenizer, resuspend the homogenate by inversion before opening the tubes.

Transfer the homogenate with one of the following methods:

• Calibration syringe method

Take 250 µl with the calibration syringe (Ref.: 355-1174) taking care to immerse the needle in the pellet of beads to avoid sampling poorly homogenized tissue fragments.

Transfer each 250 µl sample into a 2 ml Eppendorf micro test-tube or Deepwell (Ref.: 359-0132).

• Filter plate method

The transfer and the filtration are done separately using a filter plate (Ref.: 355-1179) and a Deepwell plate (Ref.: 359-0132), with either one of the two following filtration techniques.

- Vacuum technique:

Fit the Deepwell plate (Ref.: 359-0132) (the master plate) in the bottom of the vacuum manifold, place the lead of the manifold and then the filter plate (Ref.: 355-1179). Take at least 400 µl (≤ 1000 µl) with a 1000 µl tip and transfer in one well of the filter plate (Ref.: 355-1179), exclude the first 6 positions (from A1 to F1). Place a plastic sealing film on top the filter plate. Set the vacuum gauge of the pump (Ref.: 359-0350) to 25.4 cm Hg ($\pm 2.5\%$). Switch the pump on and check the gauge for correct vacuum, then open manifold valve for 1 minute ± 6 seconds. Close the valve, switch off the pump and release the vacuum from the manifold.

- Centrifugation technique:

Take at least 400 µl (≤ 1000 µl) with a 1000 µl tip and transfer in one well of the filter plate (Ref.: 355-1179) priorly fitted on a Deepwell plate (Ref.: 359-0132) (the master plate), exclude the first 6 positions (from A1 to F1). Place a plastic sealing film on top the filter plate.

Centrifuge the complete system (filter plate and Deepwell plate) for 1 min at 500 g. Taking care to keep the filtration plate securely in position on the Deepwell plate.

Note: Centrifuge must be equipped with Deepwell microplate rotor (Ref.: 359-0136), for 5804R Eppendorf centrifuge (Ref.: 359-1396).

After either technique discard the filter plate and transfer 250 µl of filtered samples into another Deepwell (the purification plate) for the manual protocol or directly place the master plate on board the NSP (refer to the TeSeE™ NSP operator's manual).

Note: At this stage, grinding tubes after homogenisation, micro test-tubes and Deepwell plate after sample transfer can be stored, closed:

	At room temperature (+18°C to +30°C) for 8 hours	At +2°C to 8°C (in ice or refrigerator) for 15 hours	At -20°C for 1 year*
Grinding tubes and micro test-tubes	Yes	Yes	Yes
Deepwell plate	Yes	Yes	No

* Frozen samples must be thawed at room temperature (+18°C to +30°C). Samples can be submitted to a maximum of 3 freezing/thawing cycles. Samples must always be homogenized by inversion before use.

4. PK treatment:

Distribute 250 µl ($\pm 10\%$) of reconstituted proteinase K solution [see paragraph 2.4] into each micro-tube or Purification plate well. Do not exceed intervals of 5 minutes for distribution of reconstituted proteinase K between the first and the last sample. Immediately homogenise closed tubes or Deepwell sealed with aluminium film 10 times by inversion. Do not exceed 2 minutes between the homogenization and the incubation at 37°C.

Incubate at 37°C $\pm 2^\circ\text{C}$ in a heating block incubator for 10 ± 1 minute.

Note: If using Deepwell, heating block must be equipped with a Deepwell rack adaptor for heating block (Ref.: 359-0134).

5. Precipitation of PrP^{Sc} with reagent B:

Remove the micro test-tubes or Deepwell plate from the heating block incubator. Open the tubes and distribute 250 µl ($\pm 10\%$) of reagent B into all micro test-tubes or Deepwell wells. Observe the same order of distribution as described in step 4. Do not exceed intervals of 2 minutes between the exit of the incubator and the homogenization. Homogenization is performed under the same conditions as in step 4.

6. Concentration of the PrP^{Sc} (centrifugation):

Within 30 minutes, after reagent B distribution and mixing : centrifuge the micro test tubes or purification plate as follows:

Centrifugation	Micro test-tubes		Deepwell plate
Speed (g)	20 000	15 000	2 000
Time (mm)	5	7	10
Temperature (°C)	20	20	4

Note: For Deepwell plate allow a 5 minute delay at 37°C or a 10 minute delay at room temperature (+18°C to +30°C) before centrifugation.

7. Sample clarifying:

Discard the supernatant by inverting the micro test-tubes over a waste container. Dry the micro test-tubes by inverting onto absorbent paper for 5 minutes.

Or load the Deepwell plate on DW40 unit (Ref.: 359-0137). Select "TSE DW" program and select number of strips to be performed. Deepwell plate wells must be dried at the end of the DW40 process, by inverting the plate on absorbent paper for 5 minutes.

Distribute 25 µl ($\pm 10\%$) of reagent C into all micro test-tubes or Deepwell wells.

Do not exceed an interval of 10 minutes between the end of the drying operation and distribution of buffer C.

Incubate immediately for 5 ± 1 minute at 100°C $\pm 5^\circ\text{C}$. Do not exceed 2 minutes between the reagent C distribution and the beginning of the incubation. Do not seal the Deepwell plate during incubation.

Note: If using Deepwell, heating block must be equipped with a Deepwell rack adaptor for heating block (Ref.: 359-0134).

Remove the micro test-tubes or the Deepwell from the incubator, and homogenate the tubes with a vortex (5 ± 2 seconds).

Samples in micro test-tubes or Deepwell can be stored for 5 hours at +2°C to +8°C or frozen for 72 hours at -20°C. Frozen samples must be thawed at room temperature (+18°C to +30°C) and homogenized with a vortex (5 ± 2 seconds).

Purified samples must be diluted with 125 µl ($\pm 10\%$) of reagent R6. Diluted samples must be homogenized with vortex (5 sec. ± 2 sec.) just before distribution into the plate (R1).

1. Remove the microplate rack and the required number of rows (R1) from the protective packaging. Replace the unused rows with the desiccated bag in the microplate sachet and hermetically close it.

2. Prepare the positive control (R4), as described in chapter 3.4.2.
3. For each series of tests and every single plate, distribute 100 µl (\pm 10%) of control/sample into wells in the following order:
 - Wells A1, B1, C1, D1: negative control (R3)
 - Wells E1, F1: positive control (R4)
 - Wells G1, H1, etc... : sample diluted with reagent (R6)
 Samples are performed in singulate.
4. Cover with adhesive film and incubate for 30 mn \pm 2 mn at 37°C \pm 2°C.
5. Prepare wash solution (R2).
6. Prepare conjugate solution (R7).
7. Remove the adhesive film, perform 3 wash cycles.
 Optimal washing conditions are obtained with PW40, PW41 or 1575 Bio-Rad plate washers with program TSE 3.
 Do not let the microplate stand for more than 5 minutes after the last wash cycle. Dry by inversion on absorbent paper before the following step.
8. Distribute 100 µl (\pm 10%) of conjugate solution (R7) into each well.
9. Cover with adhesive film and incubate 30 mn \pm 2 mn at +2°C to +8°C.
10. Prepare the enzymatic revelation solution (R8+R9).
11. Remove the adhesive film, perform 5 wash cycles.
 Optimal washing conditions are obtained with PW40, PW41 or 1575 Bio-Rad plate washers with program TSE 5.
 Do not let the microplate stand for more than 5 minutes after the last wash cycle. Dry by inversion on absorbent paper before the following step.
12. Distribute 100 µl (\pm 10%) of revelation solution (R8+R9) into each well and incubate the plate in darkness and at room temperature (+18°C to +30°C) for 30 mn \pm 2 mn. Do not use adhesive film during this incubation.
13. Add 100 µl (\pm 10%) of stop solution (R10) to each well according to the same sequence and same distribution rate as for the revelation solution.
14. Thoroughly wipe the bottom of the plate and determine the optical density at 450 nm - 620 nm (bichromatism mode) within 30 minutes after stopping the reaction (the rows must always be protected from light before reading).

Microplate washer parameters

NAME: TSE 3

EDIT mode function	PLATE	Manifold	STRIPS	Met. (Method)	MODE	CROS SW ASP.	ASP. TIME	VOLUME	OVER FLOW	LIQUID	FLOW	BOT. WASH NUMBER	BOTTOM TIME	BOT. ASP. NUMBER	SHAKE TIME	N° OF CYCLES	SOAKING	MET. INTER	N° OF KITS	KIT INTER
Main parameter	Flat01 (PW40/PW41) Flat 03 (1575)	1"9 (PW40/1575) 2"8 (PW41)	12,3,4, 5,6,7,8,9, 10,11,12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Method1	-	-	-	WASH	Plate	Yes	0.3	800	2.5	W1	0 (PW40/1575) 5 (PW41)	-	-	-	-	3	30 (PW41) 45 (PW40/1575)	0	-	-
Method2	-	-	-	BOTTOM ASP.	Plate	Yes	0.3	-	-	-	-	-	-	1	-	1	0	-	-	-

NAME: TSE 5

EDIT mode function	PLATE	Manifold	STRIPS	Met. (Method)	MODE	CROS SW ASP.	ASP. TIME	VOLUME	OVER FLOW	LIQUID	FLOW	BOT. WASH NUMBER	BOTTOM TIME	BOT. ASP. NUMBER	SHAKE TIME	N° OF CYCLES	SOAKING	MET. INTER	N° OF KITS	KIT INTER
Main parameter	Flat01 (PW40/PW41) Flat 03 (1575)	1"9 (PW40/1575) 2"8 (PW41)	12,3,4, 5,6,7,8,9, 10,11,12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Method1	-	-	-	WASH	Plate	Yes	0.3	800	2.5	W1	0 (PW40/1575) 5 (PW41)	-	-	-	-	5	30 (PW41) 45 (PW40/1575)	0	-	-
Method2	-	-	-	BOTTOM ASP.	Plate	Yes	0.3	-	-	-	-	-	-	1	-	1	0	-	-	-

PLATE NAME: FLAT 01 (PW40/PW41) - FLAT 03 (1575)

BOT. SHAPE	ASP. HOR. POS.	CENTERING	ASP. VERT. POS.	BOT. VERT. POS.	B.W. VERT. POS.	HORIZONTAL SPEED	VERTICAL SPEED	ASP. DOWNW. SPEED	DISP. UPW. SPEED	BOT. DOWNW. SPEED	BOT. UPWARD SPEED	SHAKING AMPLITUDE	SHAKING SPEED
Flat	1,4	0,3	13,5	9,5	9,5	6	8	6	9	6	9	1	9

2-7 Calculation and Interpretation of the Results

1) Calculation of the mean optical density (OD) of the negative control

$\overline{OD\ R3}$ = mean of the four OD of R3 wells

2) Calculation of the cut-off value

The cut-off value is equal to: $\overline{OD\ R3} + 0.210$

Example:

$\overline{OD\ R3} = 0.020$

Cut-off value = $0.020 + 0.210 = 0.230$

3) Condition of validation of the test

• Negative control (R3):

a) Validation of the individual negative control values:

The optical density of each individual negative control must be lower than 0.150.

However, a maximum of one individual aberrant value can be eliminated when its optical density is higher or equal to 0.150.

The test must be repeated if more than one of the negative control lies outside of this limit.

b) Homogeneity of the negative control values:

Calculate the mean of the negative controls with the individual remaining values.

Values higher than the mean of the negative controls + 40% ($\overline{OD\ R3} + 40\%$) must be eliminated.

- If one individual value is eliminated in a), one additional value can be eliminated in b).

- If no negative control value is eliminated in a), two values maximum can be eliminated in b).

The test must be repeated if more than two values of the negative control are eliminated [criteria a)+b)].

• Positive control (R4):

The mean of the positive control optical densities (R4 ODs) must be higher or equal than 1.000.

The test must be repeated if the mean of the positive control optical densities (R4 ODs) is strictly lower than this limit.

4) Interpretation of the results

Samples with an optical density lower than the cut-off value are considered to be negative according to the TeSeE™ SAP Kit instructions.

However, results situated just below the cut-off value (cut-off value - 10%) must be interpreted cautiously, and the corresponding samples should be retested in duplicate, starting from the original homogenate.

Samples with an optical density greater than or equal to the cut-off value are considered to be initially reactive according to the TeSeE™ SAP Kit instructions and should be retested in duplicate, starting from the original homogenate, before the final interpretation.

After repeating the test, the sample is considered to be positive according to the TeSeE™ SAP Kit instructions when at least one of the 2 measurements is positive (greater than or equal to the cut-off value). The sample is considered to be negative according to the TeSeE™ SAP Kit instructions when these two values are less than the cut-off value.

Samples retested in duplicate and found to be negative according to the TeSeE™ SAP Kit instructions, but for which one of the 2 values is close to the cut-off value (cut-off value - 10%) must be interpreted cautiously.

2-8 Limits of the Test

Difficulties can be encountered during the grinding step when using dehydrated samples or peripheral tissues. If necessary, the grinding step (step No.2 of the procedure) may need to be repeated several times for this type of sample.

A negative result means that the test sample does not contain any PrP^{Sc} detectable by the TeSeE™ SAP Combi Kits. However, as very low levels of PrP^{Sc} may not be detected, such a negative result does not exclude the possibility of infection.

Any sample with a reproducible positive result according to the test interpretation criteria must be confirmed in accordance with the country's national reference laboratory for TSEs or community reference laboratory in exceptional circumstances.

3 - MATERIAL REQUIRED BUT NOT SUPPLIED

- Distilled or ultrapure water.
- 20 000 ppm sodium hypochlorite (final concentration) and 1 M sodium hydroxide (final concentration).
- Absorbent paper.
- Disposable gloves.
- Protective glasses or mask with visor.

Purification step:

- 2 ml polypropylene micro test-tubes with caps and appropriate tube rack.
- Automatic or semiautomatic adjustable pipettes able to distribute volumes between 20 µl and 500 µl.
- Tissue homogenizer: Ribolyser®, TeSeE™ PRECESS 24™ or TeSeE™ PRECESS 48™.*
- Centrifuge* adapted to micro test-tubes.
- One micro test-tube heating block* thermostated at 37°C ± 2°C and one micro test-tube heating block* thermostated at 100°C ± 5°C.

For the semi-automatic purification of the sample: TeSeE™ NSP System.

Detection step:

- Automatic or semiautomatic adjustable or fixed pipettes able to distribute 50 µl, 100 µl, 200 µl and 1000 µl.
- 10 ml, 20 ml, 100 ml graduated test tubes.
- Contaminated waste containers.
- Microplate incubator thermostated at 37°C ± 2°C.
- Refrigerated chamber at +2°C to +8°C.
- Automatic or semiautomatic microplate washing system.*
- Microplate reading apparatus* (equipped with 450 nm and 620 nm filters).
- Microplate system* for the automation of the assay protocol stages. The performances of the system must conform with the requirements of the test protocol.

* Contact Bio-Rad for the list of available instruments.

4 - PRECAUTIONS

The quality of the results depends on compliance with the following good laboratory practices:

- Reagents must be stored at +2°C to +8°C.
- Do not use reagents whose shelf-life has expired.
- Do not use the reconstituted and stored at room temperature (+18°C to +30°C) proteinase K over 6 hours.
- Do not mix reagents derived from different batches of the TeSeE™ SAP Kits during the same manipulation, with the exception of generic reagents: wash solution (R2), sample diluent (R6), peroxidase substrate buffer (R8), chromogen (R9), stop solution (R10), grinding tubes, reagent A and reagent B.
- Wash solution (R2), sample diluent (R6), peroxidase substrate buffer (R8), chromogen (R9), stop solution (R10) and grinding tubes can be used with all kits from the TeSeE™ product line (TeSeE™, TeSeE™ SAP and TeSeE™ sheep/goat assays).
- Allow the reagents to adjust to room temperature (+18°C to +30°C) for 30 minutes before use.
- Thoroughly reconstitute reagents, avoiding any contamination.
- Do not perform the test in the presence of reactive vapors (acids, alkalis, aldehydes) or dust, which could alter the enzymatic activity of the conjugate.
- Only use polypropylene tubes.
- Use perfectly washed glassware, rinsed in distilled water, or preferably disposable material.
- Do not let the microplate more than 5 minutes between the end of washing and distribution of the reagents.
- The enzymatic reaction is very sensitive to all metals or metallic ions. Consequently, no metallic element must enter in contact with the various solutions containing the conjugate or the substrate.
- The revelation solution (substrate buffer + chromogen) must be colorless. The appearance of a colour few minutes after reconstitution indicates that the reagent cannot be used and must be replaced. The revelation solution should preferably be prepared with disposable plastic containers and distribution material or glassware previously washed in 1 N hydrochloric acid, rinsed in distilled water and dried. Store this solution protected from light.
- Use a new pipette tip for each sample.
- Washing of the wells is an essential step of the procedure: respect the recommended number of washing cycles and ensure that all wells are completely filled, then completely emptied. Inadequate washing can give incorrect results.
- Never use the same container and pipette to distribute the conjugate and the revelation solution.

5 - HYGIENE AND SAFETY INSTRUCTIONS

Generally, hygiene conditions, biosafety measures and good laboratory practices must be in agreement with recommendation of regular authorities of the country.

- All reagents of the kit are intended for use in “*in vitro*” diagnosis.
- Wear disposable gloves when handling reagents and samples and wash your hands thoroughly after handling them.
- Do not pipette with the mouth.
- Use polypropylene containers to avoid any wounds with broken glass.
- All the materials directly in contact with the samples and the wash solutions must be considered as contaminated.
- Avoid splashing samples or solutions containing samples.
- Contaminated surfaces must be cleaned with 20 000 ppm sodium hypochlorite solution (bleach). When the contaminating liquid is an acid, contaminated surfaces must be first neutralized with sodium hydroxide before using bleach. Surfaces must be rinsed with distilled water, dried with ethanol and wiped with absorbent paper. The material used for cleaning must be discarded in a special container for contaminated wastes.
- Samples, material and contaminated products must be eliminated after decontamination:
 - either by soaking in 1 M sodium hydroxide (final concentration) for 1 hour at room temperature (+18°C to +30°C),

- or by soaking in 20 000 ppm sodium hypochlorite solution for 1 hour at room temperature (+18°C to +30°C),
- or by autoclaving at 134°C minimum for at least 18 minutes, under 3 bars of pressure.

Note: never autoclave solutions containing sodium hypochlorite solution or reagent B.

- All operations involved in Transmissible Spongiform Encephalopathy (TSE) screening tests are subject to regulations and must be performed in an isolated, limited and controlled access laboratory devoted exclusively to this activity. A laboratory coat, overshoes, gloves, mask with visor or simple mask with safety glasses are required to ensure the operator's safety.
- Operators must receive specific training concerning the risks related to TSEs agents or prions and the validated modes of decontamination for unconventional agents. Biosafety measures must be in agreement with recommendations of regular authorities of the country.
- Avoid any contact of the substrate buffer, chromogen and stopping solution with the skin and mucous membranes.
- Neutralize and/or autoclave all wash solutions or wash wastes or any liquid containing biological samples prior to their elimination.
- Reagent B is a dangerous substance classified as nocive (> 25% alcohol) according to European legislation.
- Reagents containing 0.1% ProClin™ 300 are classified as irritating preparations according to European legislation.



Xn
(Alcohol > 25%)
(0.1% ProClin™ 300)

R : 10-22-37/38-41-43-67: Flammable. Harmful if swallowed. Irritating to respiratory system and skin. Risk of serious damage to eyes. May cause sensitisation by skin contact. Inhalation of vapour may cause drowsiness and dizziness.

S : 7/9-13-26-28-37/39-46: Keep container tightly closed and in a well ventilated place. Keep away from food, drink and animal feed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin wash immediately with plenty of water. Wear suitable protecting clothings, gloves and eye/face protection. If swallowed, seek medical advice immediately and show this container or label.

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Sample Syringe

Ref.: 355-1175

SAMPLING METHOD FOR Bio-Rad TSE SCREENING ASSAYS

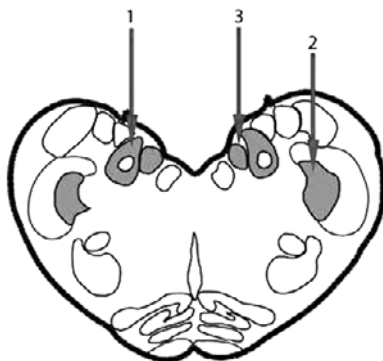
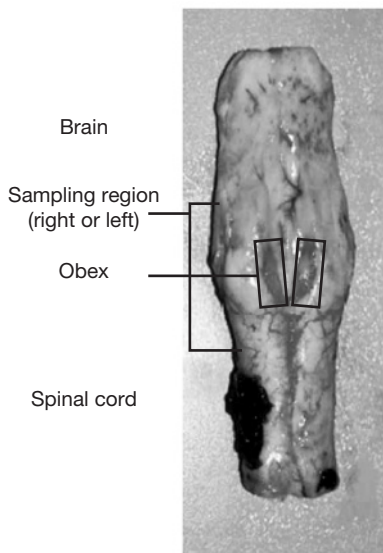


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1 - GENERAL INFORMATION

The Bio-Rad TSE screening assays are performed on a sample of 350 ± 40 mg of central nervous tissues (CNS). The specific anatomical region for detecting PrP^{Sc} in infected animals is the brain stem, more precisely in the area of the vagal nerve nucleus, in the obex region. This is the area of the brainstem where PrP^{Sc} is most concentrated.



Cross section of the brain stem at the level of the obex identifying the key target sites for diagnosis by histopathology and immunohistochemistry in BSE (nucleus of the solitary tract [1] and the nucleus of the trigeminal tract V [2]) and scrapie (dorsal nucleus of the vagus). [3]).

(Source: OIE - Manual of Diagnostic Tests and Vaccines for Terrestrial Animals)

1-1 Sample collection at the abattoir

The brain stem is easily and quickly collected with an appropriate tool or sample collection spoon, via the occipital foramen, without opening the cranial cavity.



Sample collection with the Bio-Rad collection spoon

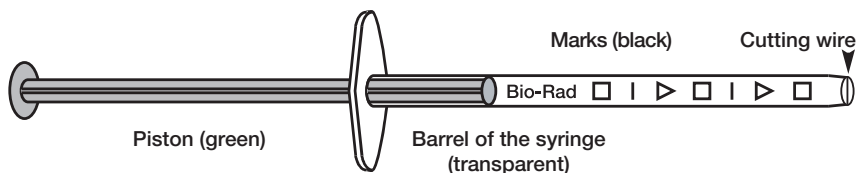
1-2 Sampling procedure at the laboratory

The whole brain stem sample is sent to the testing laboratory ensuring that appropriate bio-safety measures recommended by the regulatory authorities of the particular country are followed. In the laboratory, the appropriate amount of cerebral material is cut (scalpel blade,...) from the obex region or collected with the **Bio-Rad sample syringe (Ref: 355-1175)** which makes it possible to sample the required amount of the appropriate area quickly and safely, without any risk of sharps injuries.

The following describes the procedure to effectively collect the sample from the obex region using the Bio-Rad sample syringe, without damaging the tissue.

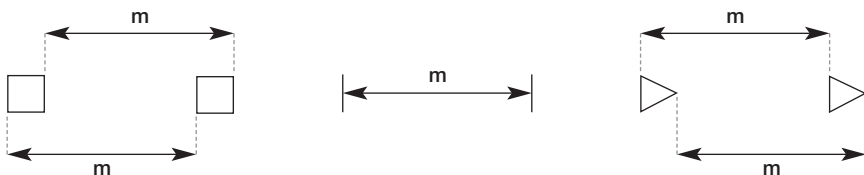
2 - Bio-Rad SAMPLE SYRINGE

The Bio-Rad sample syringe consists of a green piston and a transparent syringe barrel. The syringe barrel is labelled with a series of geometric shapes. (□ ▷ |)



3 - SAMPLE MASS REQUIRED FOR THE TEST

The sample mass should occupy the space between two symbols of the same shape which corresponds to a mass (m) of 350 +/- 40 mg.



4 - OPERATING PROCEDURE

- Take a sample syringe and pull out the green piston to approximately 1 cm from its home position then push home again.
- Firmly grasp the brain stem in one hand, using a disposable wrapper (plastic bag, glove, etc.) in order to avoid possible cross-sample contamination. The end of the brain stem should remain accessible. If the brainstem received has a cord too long, the user should trim it. Samplers should received proper training regarding the précise location of the targetted area.
- Use the other hand to position the open end of the sampling syringe on the right or left side of the caudal end of the brain stem.

Note: a complete hemi-section of brain stem with an intact obex region must remain available after sample collection for confirmatory testing.



- Insert the syringe barrel gradually into the brain stem whilst holding the green piston stationary (relative to the brain stem).

Note: While collecting the sample from the obex region, take care that the syringe barrel remains within the selected side of the brain stem.



- Stop this movement when the top of the syringe barrel has reached the upper limit of the sampling zone.
- Cut the sample core by twisting the syringe barrel through one complete turn.
- Slowly remove the sample syringe from the brain stem, taking care not to damage surrounding tissues. The remaining brain stem can be placed in its original sample container.
- Check whether there are any air gaps in the core sample collected. If needed, compress the sample core by closing the top of the syringe barrel and pushing the green piston until the air gaps have been eliminated. At the same time ensure that the tissue nearest the opening of the syringe barrel is retained.
- Holding the top of the syringe barrel still, move the green piston to the nearest symbol.
- Check that the sample core covers at least one zone corresponding to “m”, as described in the previous section of this document (sample mass required for the test).
- Take a grinding tube and remove the lid, with the sample syringe carefully depress the green piston to the next identical symbol to ensure that the correct mass of tissue (“m”) is dispensed in the grinding tube. Remember that you must move the piston to the corresponding position of the next symbol as indicated in “Sample mass required for the test”.
- Cut the sample core by gripping the top of the sample syringe against the inner edge of the grinding tube.
- Samples of extremely bad quality should be either dissected or if very autolysed pipetted up.
- The unused part of the sample core can be stored by placing the sample syringe in the original container with the remaining piece of brain stem.

5 - PRECAUTIONS/ADVICE

As for any pipetting device, Bio-Rad recommends that operators using the sample syringe should be periodically monitored, for a representative statistical population of samples taken, so ensuring that sample weights are within range.

The sample syringes are to be used only once, and then discarded in order to prevent any cross-sample contamination.

The sample must be taken with all due precautions in order to ensure that risk of contamination for operators is minimized.

The syringes used are to be discarded after being decontaminated (see Health & Safety instructions).

If the sample core does not fill the entire syringe barrel despite carrying out the procedure correctly, it is advisable to weigh the sample.

6 - HEALTH & SAFETY PROCEDURES

The hygiene conditions, bio-safety measures and good laboratory practices must comply with the guidelines of the regulatory authorities in the country.

The sample syringe is intended for use in "*in-vitro*" diagnostic procedures only.

Wear disposable gloves when handling reagents and samples, and wash your hands thoroughly after handling them.

Any equipment that has come into direct contact with the samples must be considered to have been contaminated.

Contaminated surfaces must be cleaned with 20 000 ppm sodium hypochlorite solution. When the contaminating liquid is an acid, contaminated surfaces must first be neutralized with sodium hydroxide before using sodium hypochlorite. Surfaces must be rinsed with distilled water, dried with ethanol and wiped with absorbent paper. The material used for cleaning must be discarded in a specific container for contaminated waste.

Samples, equipment and contaminated products must be discarded after decontamination using one of the following methods:

- by soaking in 1 M sodium hydroxide (final concentration) for 1 hour at room temperature (+18°C to +30°C).
- by soaking in 20 000 ppm sodium hypochlorite solution for 1 hour at room temperature (+18°C to +30°C).
- by autoclaving at a temperature of at least 134°C for a minimum of 18 minutes, at 3 bar pressure.
- **Note: never autoclave solutions containing bleach.**

All operations involved in Transmissible Spongiform Encephalopathy (TSE) screening tests are subject to local safety guidelines and must be performed in an isolated, limited and controlled-access laboratory devoted exclusively to this activity. A laboratory coat or boiler suit, overshoes, gloves (two pairs), mask with visor or simple mask with safety glasses are required to ensure the Operator's safety.

Operators must receive specific training concerning the risks related to TSE agents or prions, and the validated methods of decontamination for unconventional agents. Bio-safety measures must comply with the Guidelines of the regulatory authorities of the country concerned.



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